FLAVONOID SYSTEMATICS OF SEVEN SECTIONS OF *LUDWIGIA* (ONAGRACEAE)[1]

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ABSTRACT

Data are presented for the flavonoids of 24 species and seven sections of *Ludwigia,* all of which previously had been grouped in sect. *Myrtocarpus.* A total of eight flavonoids, three glycoflavones, and five flavonol glycosides based on quercetin was found in these species. Seven compounds, all but one acylated glycoflavone, are present in species of the revised sect. *Myrtocarpus.* The monotypic sections *Tectiflora* and *Humboldtia* have only flavonols, and the monotypic sections *Amazonia* and *Heterophylla* have only glycoflavones. Sections *Pterocaulon* and *Cinerascentes* have both flavonols and glycoflavones. Morphologically, sect. *Myrtocarpus* has the most generalized features within *Ludwigia,* which suggests that the presence of both glycoflavones and flavonols is a primitive feature for the genus as a whole. The lack of one or the other of these groups of flavonoids in four of the monotypic sections reported suggests that these sections are advanced in this feature, as does a general reduction of structural types and a reduction in the number of glycosidic substitutions in them.

As part of a comprehensive study of the flavonoids of Onagraceae, we are herein reporting results from seven sections of *Ludwigia.* As explained in earlier papers (Averett et al., 1978, 1979), the objectives of the overall study on Onagraceae are to provide an analysis of the flavonoids at the generic level for the entire family and to gain insight into the evolution of flavonoid compounds by correlating substitutional and structural changes with a phylogeny based on other systematic data. This is the first of several papers in which we shall present the results of flavonoid analyses of *Ludwigia.* The only previous report on flavonoids in *Ludwigia* is a brief summary of data for the whole genus (including that reported here) in Averett & Raven (1984).

Ludwigia is the only genus of the tribe Jussiaeeae and comprises approximately 82 species found in wet habitats in both temperate and tropical regions worldwide (Raven, 1963). *Ludwigia* appears to represent a branch of the family distinct from all other members (Eyde, 1977, 1978, 1981; Raven & Tai, 1979). It is therefore of particular interest to consider the evolution of features in this isolated evolutionary line.

This report deals with the species included in the original broad circumscription by Munz (1942; see also Raven, 1963) of sect. *Myrtocarpus,* a group of some 23 species centered in tropical and subtropical South America that appeared to be "phylogenetically central" in the genus (Raven, 1963). Based on his studies of the morphology, cytology, and crossing relationships of this complex, Ramamoorthy (1979) divided sect. *Myrtocarpus* into seven sections, commenting that the species had been grouped primarily on the basis of shared primitive characters. Subsequent work has suggested that his sect. *Michelia* is not distinct from sect. *Myrtocarpus sensu stricto,* and that *Ludwigia mexiae* (Munz) Hara is sufficiently distinct from other members of sect. *Pterocaulon* that it is best treated as the monotypic sect. *Cinerascentes* (Ramamoorthy & Zardini, 1987). Thus we now recognize seven sections in this group, delimited as follows: sect. *Myrtocarpus* with 20 species (14 examined herein), including some with the most primitive assemblages of characters in the genus; sect. *Pterocaulon* with five species that comprise a well-marked and rather homogeneous group of diploid annuals; and five monotypic sections—*Amazonia, Heterophylla, Tectiflora, Humboldtia,* and *Cinerascentes*—that are each specialized relative to sect. *Myrtocarpus* (Ramamoorthy & Zardini, 1987).

MATERIALS AND METHODS

Dried leaf materials from 24 species of seven sections of *Ludwigia* were examined for flavonoids. Approximately 120 populations in total

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and as many as 25 populations of some of the more variable species were sampled. The samples included most chromosomal races of the species concerned. Voucher specimens are listed in Table 1.

The leaf material was extracted overnight in 85% methanol and the resulting extract was examined by two-dimensional paper chromatography. Certain of the extracts were analyzed using TLC (polyamide and cellulose) as well. In some cases, the flavonoids were crudely separated on Sephadex LH 20 with a methanol/water system as described by Hiermann et al. (1978). For structural elucidation, replicate chromatograms were run and the isolated compounds cut from the paper for further purification and analysis. The quantity of leaf material varied according to usage, but approximate amounts were 0.5–1.0 g for general screening, 5–10 g for replicate chromatograms, and 20–30 g for column chromatography. Identifications of the glycosides, their aglycones, and sugars were made as previously described (Averett et al., 1978, 1979) and were compared with standard Rf values and absorption maxima (Averett, 1977). In addition, most of the aglycones and sugars were run, along with authentic reference compounds, by circular thin-layer chromatography as described by Exner et al. (1977). Base hydrolysis was employed to determine acylation but the acylating function was not determined.

RESULTS AND DISCUSSION

Eight flavonoids were found among the species sampled (Table 2): orientin (1), isoorientin (2), orientin-O-acylate (3), quercetin 3-O-rhamnoside (4), quercetin 3-O-arabinoside (5), quercetin 3-O-glucoside (6), quercetin 3-O-diglucoside (7), and quercetin 3-O-rutinoside (8). Compounds 1–3 are glycoflavones and compounds 4–8 are flavonol glycosides. All of the compounds are based on structures having two hydroxyl substituents in the B-ring.

Some infraspecific variation was found, especially in such variable species as *Ludwigia peruviana, L. elegans,* and *L. laruotteana.* Although variation was present, no flavonoid unique to any species was found in a single population. We also detected differences in concentrations of compounds between populations of some species, but made no attempt to document these differences. Interspecific variation within the larger sections is apparent but, like populational variation, is largely confined to the presence or absence of certain compounds. The more interesting variation is found in intersectional comparisons. Because of this, samples are arranged by species and grouped in their respective sections in Table 2.

Compound 3, the acylated glycoflavone, is found in only five species in three different sections, including the closely related sections *Cinerascentes* and *Pterocaulon.* Compounds 5 and 7 are the next most frequently absent, occurring in only six and seven species, respectively, and typically in low concentration when present. Compounds 1, 2, and 8 are the least variable and are found in 18, 20, and 19 of the species, respectively. Compound 2 is present in all species in which glycoflavones occur, and compound 8 is present in all species in which flavonols are found. Compounds 4 and 6 occur in 13 and 14 of the species sampled. Glycoflavones are found in 20 of the 24 species, flavonols in 19, both glycoflavones and flavonols in 15, only glycoflavones in five, and only flavonols in four.

All of the species have at least two compounds and none has more than seven. The average number of compounds per species is 5.37 for the entire group. The comparison of numbers of compounds at the sectional level is especially interesting. Species of sect. *Myrtocarpus,* which has relatively generalized features, and sect. *Cinerascentes* have an average of five compounds. The remaining sections have reduced numbers of compounds, with averages of 3.4 (*Pterocaulon*), 3.0 (*Humboldtia* and *Heterophylla*), and 2.0 (*Amazonia* and *Tectiflora*).

Flavonols are present throughout Onagraceae and glycoflavones are present in all tribes except Fuchsieae and Epilobieae (Averett & Raven, 1984). Glycoflavones are especially well represented in *Circaea* (Boufford et al., 1978; Averett & Boufford, 1985). The presence of glycoflavones is considered primitive relative to the presence of other groups of flavonoids (Harborne, 1977), and the distribution of these compounds within Onagraceae does not contradict that contention.

Except for *Ludwigia sericea,* which has only flavonols, all species examined of sect. *Myrtocarpus* and the single species, *L. mexiae,* of sect. *Cinerascentes* have both glycoflavones and flavonols. The remaining monotypic sections exhibit either glycoflavones only—sections *Heterophylla* and *Amazonia*—or flavonols only—sections *Humboldtia* and *Tectiflora.* Collectively, the species of sect. *Pterocaulon* exhibit both

TABLE 1. Voucher specimens of *Ludwigia* used for flavonoid analysis in this study. Specimens of all material deposited at MO, unless otherwise indicated.

Ludwigia section *Amazonia* Ramamoorthy

Ludwigia densiflora (Micheli) Hara. BRAZIL. RONDONIA: *Duarte 7329* (MO, RB).

Ludwigia section *Cinerascentes* Ramamoorthy & Zardini

Ludwigia mexiae (Munz) Hara. BRAZIL. PARA: *Ramamoorthy 652.*

Ludwigia section *Heterophylla* Ramamoorthy

Ludwigia inclinata (L. f.) Gómez. MEXICO. OAXACA: *Breedlove & Raven 13686.* COSTA RICA. PUNTARENAS: *Stork & Horton 8912* (US). BRAZIL. AMAPA: *Froes & Black 27732* (IAN).

Ludwigia section *Humboldtia*

Ludwigia sedoides (H. & B.) Hara. PANAMA. CANAL ZONE: *D'Arcy 12350.* BRAZIL. PARA: *Archer 8411* (RSA).

Ludwigia section *Myrtocarpus* (Munz) Hara

Ludwigia albiflora Ramamoorthy. BRAZIL. GOIAS: *Ramamoorthy 545.* MINAS GERAIS: *Ramamoorthy 427* (MO, SP).

Ludwigia bullata (Hassler) Hara. BRAZIL. MATO GROSSO DO SUL: *Ramamoorthy 610* (MO, SP), *Ramamoorthy & Vital 640* (MO, SP).

Ludwigia elegans (Camb.) Hara. BRAZIL. GOIAS: *Ramamoorthy 532, 560, 564.* MINAS GERAIS: *Ramamoorthy 403, 410, 424*; *Ramamoorthy & Vital 140*; *Ramamoorthy et al. 148, 153, 161, 168, 176, 178, 179, 181* (MO, SP), *184, 195, 299, 301, 308, 309, 319.* RIO DE JANEIRO: *Ramamoorthy et al. 291.* SAO PAULO: *Ramamoorthy 379, 384, 395*; *Ramamoorthy & Vital 112*; *Ramamoorthy et al. 196.*

Ludwigia hassleriana (Chodat) Hassler. BRAZIL. MATO GROSSE DO SUL: *Ramamoorthy 629*; *Ramamoorthy et al. 271.*

Ludwigia irwinii Ramamoorthy. BRAZIL. MINAS GERAIS: *Ramamoorthy et al. 142.* SAO PAULO: *Ramamoorthy 80*; *Munz 15406* (NY, POM, US).

Ludwigia laruotteana (Camb.) Hara. BRAZIL. GOIAS: *Ramamoorthy 419, 420.* MINAS GERAIS: *Ramamoorthy 101*; *Ramamoorthy & Vital 90*; *Ramamoorthy et al. 143, 147, 160, 172, 188, 311.* SAO PAULO: *Ramamoorthy 69.*

Ludwigia martii (Micheli) Ramamoorthy. BRAZIL. MINAS GERAIS: *Glaziou 15949* (B, C, F, P, R).

Ludwigia myrtifolia (Camb.) Hara. BRAZIL. MINAS GERAIS: *Ramamoorthy 734.*

Ludwigia nervosa (Poir.) Hara. NICARAGUA. ZELAYA: *Stevens 8275.* BRAZIL. BAHIA: *Ramamoorthy et al. 328.* DISTRITO FEDERAL: *Ramamoorthy 526.* GOIAS: *Ramamoorthy 561*; *Ramamoorthy & Vital 544.* MATO GROSSO: *Ramamoorthy 571.* MATO GROSSO DO SUL: *Ramamoorthy 605, 607.* MINAS GERAIS: *Ramamoorthy et al. 170.* SAO PAULO: *Ramamoorthy 393* (MO, SP); *Ramamoorthy & Vital 78* (MO, SP).

Ludwigia peruviana (L.) Hara. BRAZIL. MINAS GERAIS: *Ramamoorthy 366.* PARANA: *Ramamoorthy 207, 275, 281.*

Ludwigia pseudo-narcissus (Chodat) Ramamoorthy. BRAZIL. PARANA: *Ramamoorthy et al. 283.*

Ludwigia rigida (Miq.) Sandwith. SURINAM: *Pulle 475.* VENEZUELA. COJEDES: *Pittier 11711* (B, US, VEN).

Ludwigia sericea (Camb.) Hara. BRAZIL. MINAS GERAIS: *Ramamoorthy 68*; *Ramamoorthy et al. 157, 158, 159, 169.* PARANA: *Ramamoorthy et al. 215, 216, 288, 289.* SANTA CATARINA: *Ramamoorthy et al. 240.* SAO PAULO: *Ramamoorthy 444.*

Ludwigia tomentosa (Camb.) Hara. BRAZIL. BAHIA: *Ramamoorthy et al. 336.* DISTRITO FEDERAL: *Ramamoorthy 513*; *Ramamoorthy et al. 349, 351.* GOIAS: *Ramamoorthy et al. 342, 344, 345, 506.* MATO GROSSO: *Ramamoorthy & Vital 579.* MINAS GERAIS: *Ramamoorthy 163, 164, 165, 405*; *Ramamoorthy et al. 187.*

Ludwigia section *Pterocaulon* Ramamoorthy

Ludwigia decurrens Walt. NICARAGUA. ZELAYA: *Stevens 4916.* BRAZIL. MINAS GERAIS: *Ramamoorthy et al. 303, 306.* SANTA CATARINA: *Ramamoorthy et al. 258, 259.*

Ludwigia erecta (L.) Hara. MEXICO. OAXACA: *Breedlove & Raven 13669* (DS, MO). NICARAGUA. ZELAYA: *Stevens 8274.* CUBA. ORIENTE: *Ekman 6537* (S). COLOMBIA. HUILA: *Smith 1204* (GH, UC, US). SOUTHERN RHODESIA. NDANGA: *Goodier 977.*

Ludwigia filiformis (Micheli) Ramamoorthy. BRAZIL. GOIAS: *Ramamoorthy & Vital 555.* SAO PAULO: *Ramamoorthy 73.*

Ludwigia longifolia (DC.) Hara. BRAZIL. MINAS GERAIS: *Ramamoorthy & Vital 96*; *Ramamoorthy et al. 150.* SANTA CATARINA: *Ramamoorthy et al. 231, 233, 237.*

Ludwigia major (Micheli) Ramamoorthy. BRAZIL. RIO GRANDE DO SUL: *Ramamoorthy et al. 245.*

Ludwigia section *Tectiflora* Ramamoorthy

Ludwigia latifolia (Benth.) Hara. NICARAGUA. RIO SAN JUAN: *Neill 3361.* GUYANA. WEST DEMARARA: *Maguire & Fanshave 22951* (NY, U, US). PERU. SAN MARTIN: *Ferreyra 18506, Williams 7153* (F, US).

TABLE 2. Distribution of flavonoids among seven sections of *Ludwigia*. + = flavonoid detected; 0 = flavonoid not detected. Key: 1 = orientin, 2 = isoorientin, 3 = orientin-O-acylate, 4 = quercetin-3-O-rhamnoside, 5 = quercetin-3-O-arabinoside, 6 = quercetin-3-O-glucoside, 7 = quercetin-3-O-diglucoside, and 8 = quercetin-3-O-rutinoside.

	Glycoflavones			Flavonols				
	1	2	3	4	5	6	7	8
Sect. *Amazonia*								
L. densiflora	+	+	0	0	0	0	0	0
Sect. *Cinerascentes*								
L. mexiae	+	+	+	0	+	0	0	+
Sect. *Heterophylla*								
L. inclinata	+	+	+	0	0	0	0	0
Sect. *Humboldtia*								
L. sedoides	0	0	0	+	0	+	0	+
Sect. *Myrtocarpus*								
L. albiflora	+	+	0	+	0	+	0	+
L. bullata	+	+	0	+	0	+	0	+
L. elegans	+	+	0	+	+	+	+	+
L. hassleriana	0	+	0	+	0	0	+	+
L. irwinii	+	+	0	+	+	+	+	+
L. laruotteana	+	+	0	+	+	+	+	+
L. martii	0	+	0	0	0	0	+	+
L. myrtifolia	+	+	0	+	0	+	0	+
L. nervosa	+	+	0	+	0	+	0	+
L. peruviana	+	+	0	+	+	+	+	+
L. pseudo-narcissus	+	+	0	0	0	0	0	+
L. rigida	+	+	0	0	0	0	0	+
L. sericea	0	0	0	+	0	+	0	+
L. tomentosa	+	+	0	+	+	+	0	+
Sect. *Pterocaulon*								
L. decurrens	+	+	0	0	0	+	+	+
L. erecta	+	+	+	0	0	0	0	0
L. filiformis	0	0	0	+	0	+	0	+
L. longifolia	+	+	+	0	0	0	0	0
L. major	+	+	+	0	0	0	0	0
Sect. *Tectiflora*								
L. latifolia	0	0	0	0	0	+	0	+

glycoflavones and flavonols, but only one species, *L. decurrens*, has both classes of compounds. One species, *L. filiformis*, has only flavonols, and the remaining three species have only glycoflavones. Thus, if sect. *Pterocaulon* is a monophyletic group derived from sect. *Myrtocarpus*, as is indicated from morphological studies, then within this section of five species one has lost the ability to produce glycoflavones and three to produce flavonols; that, at least, would be the most parsimonious explanation.

The five monotypic sections we are considering here do not appear to be more closely related to sect. *Myrtocarpus*, on the basis of their overall characteristics, than they do to any other part of *Ludwigia*. Additionally, they differ in many morphological features one from another, and there is no evidence of a direct relationship between any two. The overall similarity of flavonoids between sections *Tectiflora* and *Humboldtia* and between sections *Amazonia* and *Heterophylla* could not, then, be taken as an indication of relationship between those taxa. Rather, the similarity of flavonoids between these

groups seems to reflect parallel and independent loss of particular classes of flavonoids and/or individual compounds, a trend that has characterized the evolution of the genus overall. A further evaluation of their relationships, which must be multidimensional, would need to take into account the remainder of the genus. It does appear, in terms of admittedly largely plesiomorphic characteristics, that sections *Pterocaulon* and *Cinerascentes* are more clearly related to sect. *Myrtocarpus* than are the others.

In summary, our analysis has revealed a pattern of loss of individual flavonoids and groups of flavonoids in the seven sections of *Ludwigia* that we have considered in this paper. Further resolution of the relationships of these species must await more detailed study.

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